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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/623,578	07/22/2003	Lars Blank	030307-0217	6542

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EXAMINER	
ARIANI, KADE	

ART UNIT	PAPER NUMBER
1651	

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08/19/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/623,578	BLANK ET AL.	
	Examiner	Art Unit	
	KADE ARIANI	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05/28/2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *31-47* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *31-47* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

The amendment filed on May 28, 2008, has been received and entered.

Claims 31-47 are pending in this application and were examined on their merits.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 31-47 are rejected under 35 U.S.C. 103(a) as being unpatentable over Jensen & Hammer (Biotechnol Bioeng, 1998, Vol. 58, p. 191-195) and Jensen et al. (PNAS, 1993, Vol. 90, p.8068-8072) in view of Ward et al. (Journal of Bacteriology, 2000, Vol. 182, No. 11, p.3239-3246), and further in view of de Vos (Antonie van Leeuwenhoek 1996, Vol.70, p.223-242).

Claims 31-47 are drawn to a culture of lactic acid bacterial cells, reduced glycolytic flux and, and under aerobic conditions, a respiratory metabolism, whereby said culture displays a yield of biomass exceeding that obtainable from substrate-level phosphorylation, the reduced glycolytic flux is provided by introducing mutations in said cells to generate a lower rate of metabolism of the carbon source and respiratory metabolism is provided by introducing manipulations to said cells to produce an

increased yield of ATP in said cells via oxidative phosphorylation when said cells are propagated in the presence of a terminal electron acceptor, starter culture comprising the lactic acid bacterial culture, the composition is frozen, a bacterial nutrient or cryoprotectant, 10^4 to 10^{12} CFU/g, cells contain a cytochrome (at least 0.1 ppm on a dry matter basis), and two or more different lactic acid bacterial strains.

Applicants state that "glycolytic flux" relates to the consumption of a carbon source and that "reduced glycolytic flux" relates to a flux in a cell which is reduced relative to the flux in cells cultivated under aerobic conditions in the presence of a porphyrin compound and in excess amounts of lactose or glucose (specification page 14, lines 9-12). As the definitions are not explicit, i.e. applicant used the phrasing "relates to," the broadest reasonable interpretation of "reduced glycolytic flux" would be the reduction of the ability to consume any carbon source – this is the claim construction the examiner has used to examine the claims.

Jensen & Hammer teach culture of lactic acid bacterial cells (*Lactococcus lactis)*. Jensen & Hammer do not specifically mention a reduced glycolytic flux. Jensen & Hammer however do teach metabolic engineering strategies to increase the productivity of microbial bioreactors, expression systems and strategies to introduce mutations and allow the modulation of gene expression in *Lactococcus lactis*. Jensen & Hammer teach it is necessary to perform metabolic optimization to modulate the expression of the relevant gene around the normal expression level and determine to optional expression level, for instance as the level that maximize a particular flux or yield (p.192, 1st column, p.195, 1st column, 3rd paragraph). The culture claimed would have been obvious at the

time the invention was made because it would have been obvious to metabolically engineer the lactic acid bacteria of Jensen & Hammer to have a reduced glycolytic flux and respiratory metabolism under aerobic conditions because Jensen et al. (2nd Jensen PNAS) teach introducing mutations to generate a lower rate of metabolism of the carbon source (reduced glycolytic flux), overexpression of H^+ ATPase (during aerobic growth with glucose as growth substrate) results in an increased [ATP/ [ADP] ratio, the negative control on growth rate, and the production of building blocks for more biomass (increased biomass) (p. 8072 1st paragraph), and further teach flux control should rather reside in substrate transport. Please note that in organisms, which contain a respiratory chain, the primary role of H^+ ATPase is to synthesize ATP driven by the proton gradient that results from respiration, when these organisms are supplied with an electron acceptor. Jensen et al. (2nd Jensen PNAS) teach H^+ ATPase is a key enzyme in the mechanism of cellular free-energy metabolism. Jensen et al. teach mutations in the *lac* operon to allow for ready control and monitoring of the expression of the H^+ ATPase (*atp* operon) (see Material & methods).

Further motivation to do the genetic modification described above is provided by Ward et al. who teach the relationship between the reduced glycolysis and increase in the biomass as a result of ATP production via substrate level phosphorylation under aerobic conditions. Ward et al. teach in *Enterococcus faecalis* culture, under aerobic condition, expression of a gene cluster involved in the metabolism of a growth substrate result in formation of ATP via substrate level phosphorylation a marked increase in biomass, expression of the gene cluster is repressed in the presence of a more readily

metabolizable carbon source such as glucose (see Abstract & p. 3242, 2nd column, 2nd paragraph).

Even further motivation to do the genetic modification discussed above is provided by De Vos who teaches the main factor causing the metabolic inflexibility of lactic acid bacteria is the absence of functional electron transport chain, this prevents the generation of energy by the reduction of external electron acceptors, thereby limiting the number of catabolic pathways that provide energy (p. 223, 2nd column, lines 1-6),

De Vos teaches only two ways are known by which LAB generate metabolic energy, the simplest is substrate level phosphorylation, and a second indirect way for the generation of metabolic energy in LAB is from the conversion of a solute gradient into an electrochemical gradient of protons (by secondary transporters), the proton gradient is used to generate ATP via the membrane-located ATPase (p.224, 11stcolumn, lines 1-4).

De Vos teaches physiological studies have shown that lactic acid bacteria show greater metabolic potential when the reduced cofactors (from which NADH is the most important) are regenerated by exogenous electron acceptors (p. 236, column 1, 2nd paragraph).

De Vos also teaches it is evident that lactic acid bacteria have very flexible metabolism and that metabolic engineering allows for the further expansion of the potential of lactic aid bacteria as starter cultures in products or as cell factories in fermentors. De Vos further teaches the modulation of concentration of important

substrates such as [NADH]/ [NAD+] and [ATP [/ [ADP] ratios is now feasible in *L. lactis* by using well-controlled promoters (p.238, 2nd column, lines 5-10).

The cited references do not teach a cryoprotectant, 10^4 to 10^{12} CFU/g, and cells contain at least 0.1 ppm on a dry matter basis cytochrome. However, routine experimentation is widely used by one of ordinary skill in the art to determine optimum or workable ranges of particular parameters such as pH, temperature, concentration of the enzyme or its substrate. "[W] here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955) (MPEP Chapter 2100 - p.141). Also, at the time the invention was made adding glycerol (cryoprotectant) prior to freeze-drying the cells to enhance the viability of bacterial cells during storage was very well known in the art.

Therefore, a culture of lactic acid bacteria with a reduced glycolytic flux and respiratory metabolism under aerobic conditions would have been obvious at the time the invention was made because it would have been obvious to one of ordinary skill in the art to manipulate (to introduce mutations to) LAB cells to have the predictable results of reduced glycolytic flux (lower metabolic rate of a carbon source) and a respiratory metabolism by an increased yield of ATP (via oxidative phosphorylation in the presence of a terminal electron acceptor) or the reasons set forth above.

Response to Arguments

Applicant's arguments filed on 05/28/2008 have been fully considered but they are not persuasive.

Applicant argues that in Jensen the intracellular ATP/ADP ratio is positive, while the desired effect of the claimed invention is to reach a reduced ATP/ADP ratio, thus Jensen 2, does not provide a reduced glycolytic flux since the uptake of glucose is not limited, and that Jensen 2 describes an increased glycolytic flux.

Applicant argues that Jensen2 does not suggest or motivate one skill in the art to produce a reduced glycolytic flux in combination with a respiratory metabolism.

However, claim 42 requires "…introducing manipulation to said cells to produce an increased yield of ATP in said cells via oxidative phosphorylation...", and Jensen 2 teach overexpression of H^+-ATPase enzyme results in an increased ATP/ADP ratio, and high ATP/ADP ratio which means higher ATP with respect to ADP. Thus Jesnen2 teach introducing manipulation to said cells to produce an increased yield of ATP in said cells via oxidative phosphorylation.

Furthermore, Jensen2 (PNAS) teach there are two routes that synthesize ATP and have control over the total flux, once much of the oxidative phosphorylation capacity has been eliminated, the other route, substrate-level phosphorylation becomes dominant and the route of oxidative phosphorylation has little control on total flux. Jensen2 teach when succinate is the growth substrate (when there is much less substrate-level phosphorylation), it was observed that the expression of atp operon was increased above the wild-type level, the overexpression of H^+-ATPase enzyme results in an increased ATP/ADP ratio, and lower concentration of the ADP (at high ATP/ADP

ratio) can lead to inhibition of phosphofructokinase (p.8071 2nd column last paragraph and p.8072 1st column 1st paragraph). It must be noted that phosphofructokinase is the key enzyme of glycolysis, thus, Jensen2 teach reduced glycolytic flux, and motivate one skill in the art to produce a reduced glycolytic flux in combination with a respiratory metabolism.

. Applicant argues that the cited references, either alone or in combination, fail, to suggest the claimed lactic acid bacteria in an increased yield of biomass.

However, as mentioned immediately above, De Vos teaches the presence of the membrane-located ATPase in lactic acid bacteria that use proton gradient used to generate ATP (p.224, 11stcolumn, lines 1-4). Thus, a person of ordinary skill in the art would have been motivated to combine the prior art teachings and use Jensen et al. teachings to introduce manipulations to lactic acid bacterial cells to produce a reduced glycolytic flux in combination with a respiratory metabolism.

Furthermore, as mentioned immediately above, De Vos also teaches lactic acid bacteria have very flexible metabolism and that metabolic engineering allows for the further expansion of the potential of lactic aid bacteria as starter cultures, and the availability of well-controlled promoters for the modulation of concentration of important substrates such as [NADH]/ [NAD+] and [ATP [/ [ADP] ratios in LAB cells.

Therefore, all the claimed elements were known in the prior art and a person of ordinary skill could have combined the elements by known methods and the combination would have yielded predictable results to one of ordinary skill in the art at the time the invention was made.

Conclusion

No claims are allowed.

THIS ACTION IS MADE FINAL. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kade Ariani whose telephone number is (571) 272-6083. The examiner can normally be reached on 9:00 am to 5:30 pm EST Mon-Fri.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on (571) 272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status

information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Leon B Lankford/
Primary Examiner, Art Unit 1651

Kade Ariani
Examiner
Art Unit 1651